Soyasaponin and α-tomatine inhibit *in vitro* bioaccessibility of cholesterol

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Abstract

Saponins are a structurally diverse family of secondary plant metabolites that confer protection against pathogens and predators. These compounds consist of triterpene or steroidal nuclei (aglycones) covalently linked to either mono- or oligo-saccharides. In vitro and several in vivo studies have suggested that these amphipathic compounds possess anti-carcinogenic, hypo-lipemic, hypo-cholesterolemic and immuno-enhancing activities. Limited investigations indicate that saponins are poorly absorbed, but their potential impact on digestion and gut health remains unknown. We have initiated studies to compare the effects of saponins extracted from soy, tomato, chickpea, fenugreek and a commercial mixture of phytosterols (positive control) on cholesterol absorption and metabolism, as well as on interactions between microflora and the gut epithelium. Pilot studies demonstrate significant differences in the ability of equimolar concentrations of saponins from these sources to inhibit micellarization of cholesterol from a food matrix during simulated digestion with α-tomatine >> phytosterols > soyasaponin. Removal of the oligosaccharide from tomatine to generate tomatidine markedly decreased activity. Saponins from soya and tomato, as well as their aglycones, and phytosterols all slightly, but significantly, impaired transfer of cholesterol from micelles to human intestinal Caco-2 cells. These preliminary observations suggest that saponins from crops important to Ohio economic status can modulate the bioavailability of dietary cholesterol.

Materials and Methods

Simulated digestion and micellarization of cholesterol in vitro. In vitro digestion was conducted using yogurt as the food matrix as described elsewhere.[4] Test compounds included soyasaponin I, soyasapogenol B (aglycone), α-tomatine, and tomatidine (aglycone). The impact of equimolar concentrations (90-93 μM) of test compounds added to the matrix on the micellarization of cholesterol (14 μM with 25 nCi ^{14}C-cholesterol in 100 μL sunflower oil/25 mL reaction) during small intestinal phase of digestion was compared to micellarization during digestion of the matrix containing cholesterol alone. Commercial phytosterol (containing 14 μM cholesterol) served as a positive control as it is known to inhibit micellarization of cholesterol.[5] The ratio of saponins/phytosterol to cholesterol in all test samples was approximately 6.5. After digestion, an aliquot of digesta was centrifuged to obtain the aqueous fraction. ^{14}C-cholesterol was quantified in digesta and filtered aqueous fractions of the samples via scintillation counting to determine the extent of micellarization of cholesterol.

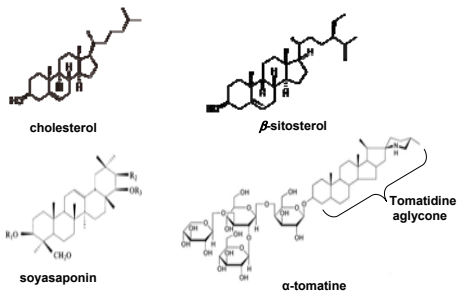
Preparation of Tween Micelles. Cholesterol, phytosterols, soyasaponin, sapogenol, a-tomatine, and tomatidine were each transferred into 11 mL vials. DMSO was added to dissolved saponins, and 1 mL of Tween 40 (20% in acetone) was added (final concentration of DMSO < 0.01% and Tween was 0.002 % in test media). After mixing, the solution was dried down under N_2 gas. Basal media was added and sonicated for 30 minutes to form cholesterol and saponin micelles, which were sterile filtered (0.22 μM filters) and diluted at indicated concentrations for exposure to Caco-2 cells. Tween micelles were used to investigate cytotoxicity. To investigate cholesterol uptake by Caco-2 cells, media containing either cholesterol, phytosterols, soyasaponin, sapogenol, a-tomatine, or tomatidine was prepared as previously described except 5nCi ^{14}C/mL was added in cholesterol vial before the mixture was dried under N_2 gas. One mililitre of cholesterol micelles and1 mL of test compound micelles were added to the monolayer (total 2 mL per well). Final ratio of cholesterol to each test compound was 1:6.5 in test media.

Cytotoxicity. Differentiated cultures of Caco-2 cells(HTB37) were exposed to a range of concentrations of soyasaponin, soyasapogenol, α-tomatine, and tomatidine for 48 h. Cytotoxicity was assessed by morphological appearance as compared to control cultures. Relative cell number was estimated by Sulforhodamine B (SRB) assay.[6]

Cholesterol uptake by Caco-2 cells. Caco-2 cells (HTB 37; passage 38 at 14d post-confluency) were incubated in DMEM containing cholesterol and test compounds (at molar ratio of 1:6.5) and incubated in 95% air:5%CO_2 for 4 h. Monolayers were washed once with ice cold PBS containing albumin (2 g/L) and once with cold PBS alone. Lysis buffer (0.5 mL) was added to each well and shaken on a gyratory shaker for 10 minutes until the monolayer lifted. The cell material was sonicated for 10 seconds and analyzed *via* scintillation counting to determine cholesterol uptake.

Statistical analysis. Data are expressed as means ± SEM and were analyzed by one-way ANOVA and Tukey's Post Hoc pair wise comparison using Minitab for Windows (Minitab v15.1; State College, PA). Differences of P<0.05 were considered significant.

Introduction



Saponins are a diverse family of amphiphilic compounds that consists of triterpene or steroidal nuclei covalently linked to mono- or oligo-saccharides and are found in a variety of edible plants such as soya, tomato, chickpea and fenugreek.[1] Saponins are structurally similar to phytosterols (e.g., β-sitosterol). The hypocholesterolemic activity of phytosterols and phytostanols has resulted in their increased incorporation into food products targeted to decrease plasma cholesterol levels.[5] Limited data from in vitro and animal studies have suggested that soyasaponins also possess hypocholesterolemic activity.

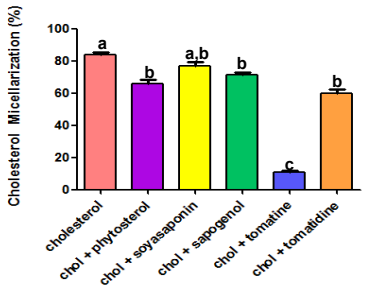
Cholesterol is both absorbed from the diet and synthesized in tissues. It is generally unknown if the hypocholesterolemic activity of saponins is due to adverse effects on cholesterol absorption or post-absorptive effects on utilization. Because saponins are poorly absorbed, adverse effects on the absorption of cholesterol and its bile salt derivatives are likely. The planar steroid ring nucleus of saponins likely interacts with the planar rings of cholesterol and bile salts to impede their respective absorption and re-absorption. This effect results in decreasing plasma and tissue concentrations of cholesterol.[1,2, 3] Thus, saponins may contribute to the health-promoting properties of foods such as soya and tomato.

Objective

The specific aim of these pilot studies was to determine the effect of saponins isolated from several plant foods on the micellarization of cholesterol during simulated gastric and small intestinal digestion and on the uptake of cholesterol by Caco-2 cells human intestinal cells.

Results

Impact on Saponins on Cholesterol Micellarization during Simulated Digestion

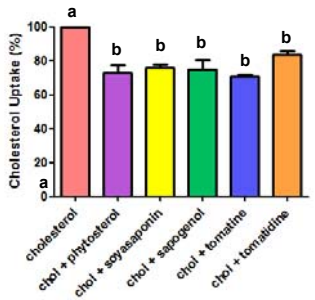


α-tomatine is a potent inhibitor of cholesterol micellarization. Yogurt containing cholesterol without or with indicated test compounds was subjected to simulated digestion. The amount of cholesterol in digesta that partitioned into micelles (filtered aqueous fraction) was measured. Data mean ± SEM of n=≥6 with significant differences (p<0.05) denoted by different superscripts.

Cytotoxicity of saponins for Caco-2 cells is proportional to their ability to inhibit micellarization of cholesterol

Compound	Concentration	Observation after 48 h
soyasaponin	≥ 185 μM	No cytotoxicity
sapogenol	≥ 270 μM	No cytotoxicity
α-tomatine	> 25 μM	Cytotoxic
tomatidine	≥ 35 μM	Cytotoxic

Impact on Cholesterol Uptake



Saponins from soya and tomato inhibit cholesterol uptake by Caco-2 cells. Data are means ± SEM (n=5-6) with significant differences (p<0.05) denoted by different superscripts above the error bars

Summary

Cholesterol micellarization

- Phytosterol mixture inhibited cholesterol micellarization ~ 21%, whi similar to inhibition observed in previous studies[5]
- Sapogenol and tomatidine inhibited micellarization of cholesterol to similar extent as phytosterols
- α-tomatine was the most potent of the tested saponin for inhibition cholesterol micellarization (~87%)
- Increased cytotoxicity of α-tomatine in comparison to soyasaponin corresponds with greater apparent affinity for cholesterol as observe robust inhibitory effect on micellarization during digestion

Cholesterol Uptake

- Soyasaponin, sapogenol, α-tomatine, and tomatidine exhibited sim although limited, inhibitory activity of cholesterol uptake

Cytotoxicity

- α-tomatine is highly toxic to Caco-2 cells in comparison to soyasap The greater toxicity corresponds with greater apparent affinity for cholesterol as observed for robust inhibitory effect on micellarization during digestion

Future Studies

- Investigate the effects of saponins from chickpea and fenugreek on cholesterol micellarization and uptake by Caco-2 cells
- Determine the lowest concentration of α-tomatine that significantly inhibits cholesterol micellarization to determine if such amounts can be selected fo varieties of this fruit
- Examine possible effects of saponins on esterification of cholesterol in enterocytes
- Examine effects of saponins on expression of virulence genes in pathoge Salmonella, as well as their adherence, invasion, and survival in enterocyte

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